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DB=USPT,PGPB,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ

<u>L12</u>	vaccine and l5	64	<u>L12</u>
<u>L11</u>	l8 and l5	195	<u>L11</u>
<u>L10</u>	l8 same l5	5	<u>L10</u>
<u>L9</u>	L8 with l5	2	<u>L9</u>
<u>L8</u>	cytokine or immune response or immunogen or antigen or immunostimulatory	113512	<u>L8</u>
<u>L7</u>	l6 same l5	13	<u>L7</u>
<u>L6</u>	gene therapy	25017	<u>L6</u>
<u>L5</u>	L4 with l3	215	<u>L5</u>
<u>L4</u>	dna or nucleic or plasmid or polynucleotide	187568	<u>L4</u>
<u>L3</u>	L2 with l1	343	<u>L3</u>
<u>L2</u>	cationic lipid or cationic amphiphile or cationic liposome	5482	<u>L2</u>
<u>L1</u>	encapsulat\$	150178	<u>L1</u>

END OF SEARCH HISTORY

WEST**Freeform Search****Database:**

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Term:

vaccine and 15

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10

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1

Generate: Hit List Hit Count Side by Side Image**Search History****DATE:** **Monday, March 17, 2003** [Printable Copy](#) [Create Case](#)

WEST

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L11: Entry 22 of 195

File: PGPB

Dec 19, 2002

DOCUMENT-IDENTIFIER: US 20020192651 A1

TITLE: Method of preventing aggregation of a lipid: nucleic acid complex

Summary of Invention Paragraph (16):

[0014] In one embodiment, a plasmid is combined with cationic lipids in a detergent solution to provide a coated plasmid-lipid complex. The complex is then contacted with non-cationic lipids to provide a solution of detergent, a plasmid-lipid complex and non-cationic lipids, and the detergent is then removed to provide a solution of serum-stable plasmid-lipid particles, in which the plasmid is encapsulated in a lipid bilayer. The particles thus formed have a size of about 50-150 nm.

Summary of Invention Paragraph (17):

[0015] In another embodiment, serum-stable plasmid-lipid particles are formed by preparing a mixture of cationic lipids and non-cationic lipids in an organic solvent; contacting an aqueous solution of plasmid with the mixture of cationic and non-cationic lipids to provide a clear single phase; and removing the organic solvent to provide a suspension of plasmid-lipid particles, in which the plasmid is encapsulated in a lipid bilayer, and the particles are stable in serum and have a size of about 50-150 nm.

Brief Description of Drawings Paragraph (41):

[0065] FIGS. 41A, 41B and 41C illustrate the encapsulation of plasmid DNA in a lipid vesicles by the detergent dialysis method using different cationic lipids.

Detail Description Paragraph (66):

[0134] The present invention provides a method of preparing serum-stable plasmid-lipid particles in which the plasmid is encapsulated in a lipid-bilayer and is protected from degradation. Additionally, the particles formed in the present invention are preferably neutral or negatively-charged at physiological pH. For in vivo applications, neutral particles are advantageous, while for in vitro applications the particles are more preferably negatively charged. This provides the further advantage of reduced aggregation over the positively-charged liposome formulations in which a nucleic acid can be encapsulated in cationic lipids.

Detail Description Paragraph (67):

[0135] The particles made by the methods of this invention have a size of about 50 to about 150 nm, with a majority of the particles being about 65 to 85 nm. The particles can be formed by either a detergent dialysis method or by a modification of a reverse-phase method which utilizes organic solvents to provide a single phase during mixing of the components. Without intending to be bound by any particular mechanism of formation, FIG. 3 depicts a detergent dialysis approach to the formation of the plasmid-lipid particles. With reference to FIG. 3, a plasmid or other large nucleic acid is contacted with a detergent solution of cationic lipids to form a coated plasmid complex. These coated plasmids can aggregate and precipitate. However, the presence of a detergent reduces this aggregation and allows the coated plasmids to react with excess lipids (typically, non-cationic lipids) to form particles in which the plasmid is encapsulated in a lipid bilayer. As noted above, these particles differ from the more classical liposomes both in size (liposomes being typically 200-400 nm) in that there is little or no aqueous medium encapsulated by the particle's lipid bilayer. The methods described below for the formation of plasmid-lipid particles using organic solvents follow a similar scheme.

Detail Description Paragraph (166):

[0234] Gene therapy relies on the efficient delivery of therapeutic genes to target cells. Most of the somatic cells that have been targeted for gene therapy, e.g., hematopoietic cells, skin fibroblasts and keratinocytes, hepatocytes, endothelial cells, muscle cells and lymphocytes, are normally non-dividing. Retroviral vectors, which are the most widely used vectors for gene therapy, unfortunately require cell

division for effective transduction (Miller et al., Mol. Cell. Biol. 10:4239-4242 (1990)). This is also true with other gene therapy vectors such as the adeno-associated vectors (Russell et al., Proc. Natl. Acad. Sci. USA 91: 8915-8919 (1994); Alexander et al., J. Virol. 68: 8282-8287 (1994); Srivastava, Blood Cells 20: 531-538 (1994)). Recently, HIV-based vectors has been reported to transfect non-dividing cells (CITE). Nonetheless, the majority of stem cells, a preferred target for many gene therapy treatments, are normally not proliferating. Thus, the efficiency of transduction is often relatively low, and the gene product may not be expressed in therapeutically or prophylactically effective amounts. This has led investigators to develop techniques such as stimulating the stem cells to proliferate prior to or during gene transfer (e.g., by treatment with growth factors) pretreatment with 5-fluorouracil, infection in the presence of cytokines, and extending the vector infection period to increase the likelihood that stem cells are dividing during infection, but these have met with limited success.

Detail Description Paragraph (311):

[0353] This example illustrates the encapsulation of plasmid DNA in a lipid vesicles by the detergent dialysis method using different cationic lipids. The dialysis method is as described previously for DODAC (EXAMPLE 1). The amount of plasmid entrapped with different mol % of the various cationic lipids was determined by DEAE Sepharose chromatography (described in EXAMPLE 2). The entrapment efficiency was similar for all cationic lipids tested with approximately 50 to 60% of plasmid DNA. The cationic lipid concentration required in the formulation for optimal plasmid encapsulation was 6.5% for DOTMA, DSDAC and DODMA-AN in FIG. 41(a); 8% DODAC and DMRIE in 41(b); DCchol in 41(c).

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L11: Entry 154 of 195

File: USPT

Nov 2, 1999

DOCUMENT-IDENTIFIER: US 5976567 A

TITLE: Lipid-nucleic acid particles prepared via a hydrophobic lipid-nucleic acid complex intermediate and use for gene transfer

Brief Summary Text (16):

In one embodiment, a plasmid is combined with cationic lipids in a detergent solution to provide a coated plasmid-lipid complex. The complex is then contacted with non-cationic lipids to provide a solution of detergent, a plasmid-lipid complex and non-cationic lipids, and the detergent is then removed to provide a solution of serum-stable plasmid-lipid particles, in which the plasmid is encapsulated in a lipid bilayer. The particles thus formed have a size of about 50-150 nm.

Brief Summary Text (17):

In another embodiment, serum-stable plasmid-lipid particles are formed by preparing a mixture of cationic lipids and non-cationic lipids in an organic solvent; contacting an aqueous solution of plasmid with the mixture of cationic and non-cationic lipids to provide a clear single phase; and removing the organic solvent to provide a suspension of plasmid-lipid particles, in which the plasmid is encapsulated in a lipid bilayer, and the particles are stable in serum and have a size of about 50-150 nm.

Drawing Description Text (42):

FIGS. 41A, 41B and 41C illustrate the encapsulation of plasmid DNA in a lipid vesicles by the detergent dialysis method using different cationic lipids.

Detailed Description Text (65):

The present invention provides a method of preparing serum-stable plasmid-lipid particles in which the plasmid is encapsulated in a lipid-bilayer and is protected from degradation. Additionally, the particles formed in the present invention are preferably neutral or negatively-charged at physiological pH. For in vivo applications, neutral particles are advantageous, while for in vitro applications the particles are more preferably negatively charged. This provides the further advantage of reduced aggregation over the positively-charged liposome formulations in which a nucleic acid can be encapsulated in cationic lipids.

Detailed Description Text (66):

The particles made by the methods of this invention have a size of about 50 to about 150 nm, with a majority of the particles being about 65 to 85 nm. The particles can be formed by either a detergent dialysis method or by a modification of a reverse-phase method which utilizes organic solvents to provide a single phase during mixing of the components. Without intending to be bound by any particular mechanism of formation, FIG. 3 depicts a detergent dialysis approach to the formation of the plasmid-lipid particles. With reference to FIG. 3, a plasmid or other large nucleic acid is contacted with a detergent solution of cationic lipids to form a coated plasmid complex. These coated plasmids can aggregate and precipitate. However, the presence of a detergent reduces this aggregation and allows the coated plasmids to react with excess lipids (typically, non-cationic lipids) to form particles in which the plasmid is encapsulated in a lipid bilayer. As noted above, these particles differ from the more classical liposomes both in size (liposomes being typically 200-400 nm) in that there is little or no aqueous medium encapsulated by the particle's lipid bilayer. The methods described below for the formation of plasmid-lipid particles using organic solvents follow a similar scheme.

Detailed Description Text (165):

Gene therapy relies on the efficient delivery of therapeutic genes to target cells. Most of the somatic cells that have been targeted for gene therapy, e.g., hematopoietic

cells, skin fibroblasts and keratinocytes, hepatocytes, endothelial cells, muscle cells and lymphocytes, are normally non-dividing. Retroviral vectors, which are the most widely used vectors for gene therapy, unfortunately require cell division for effective transduction (Miller et al., Mol. Cell. Biol. 10:4239-4242 (1990)). This is also true with other gene therapy vectors such as the adeno-associated vectors (Russell et al., Proc. Natl. Acad. Sci. USA 91: 8915-8919 (1994); Alexander et al., J. Virol. 68: 8282-8287 (1994); Srivastava, Blood Cells 20: 531-538 (1994)). Recently, HIV-based vectors has been reported to transfect non-dividing cells (CITE) Nonetheless, the majority of stem cells, a preferred target for many gene therapy treatments, are normally not proliferating. Thus, the efficiency of transduction is often relatively low, and the gene product may not be expressed in therapeutically or prophylactically effective amounts. This has led investigators to develop techniques such as stimulating the stem cells to proliferate prior to or during gene transfer (e.g., by treatment with growth factors) pretreatment with 5-fluorouracil, infection in the presence of cytokines, and extending the vector infection period to increase the likelihood that stem cells are dividing during infection, but these have met with limited success.

Detailed Description Text (316):

This example illustrates the encapsulation of plasmid DNA in a lipid vesicles by the detergent dialysis method using different cationic lipids. The dialysis method is as described previously for DODAC (EXAMPLE 1). The amount of plasmid entrapped with different mol % of the various cationic lipids was determined by DEAE Sepharose chromatography (described in EXAMPLE 2). The entrapment efficiency was similar for all cationic lipids tested with approximately 50 to 60% of plasmid DNA. The cationic lipid concentration required in the formulation for optimal plasmid encapsulation was 6.5 % for DOTMA, DSDAC and DODMA-AN in FIG. 41(a); 8% DODAC and DMRIE in 41(b); DCchol in 41(c).

WEST

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L12: Entry 42 of 64

File: USPT

Oct 30, 2001

DOCUMENT-IDENTIFIER: US 6309569 B1
TITLE: Encapsulation of bioactive agents

Detailed Description Text (59):

The present invention still further provides a composition comprising mixtures of microparticles of less than 10 microns in diameter which contain an external polymer shell and an internal aqueous solution of a bioactive agent, said composition comprising a first microparticle wherein the polymer is PLG of molecular weight less than 50 kD and a second microparticle wherein the polymer is PLG of molecular weight more than 70 kD. Very complex mixtures of individual types of polymer particles can be mixed and used as combination vaccines or therapies.

Other Reference Publication (5):

Chen, S.C. et al., "Protective Immunity Induced by Oral Immunization with a Rotavirus DNA Vaccine Encapsulated in Microparticles," *J. Virol.* 72:5757-5761 (Jul. 1998).

Other Reference Publication (7):

Eldridge, J.H. et al., "Controlled Vaccine Release in the Gut-Associated Lymphoid Tissues. I. Orally Administered Biodegradable Microspheres Target the Peyer's Patches," *J. Cont. Rel.* 11:205-214 (1990).

Other Reference Publication (15):

Jones, D.H. et al., "Poly(DL-lactide-co-glycolide) - encapsulated plasmid DNA elicits systemic and mucosal antibody responses to encoded protein after oral administration," *Vaccine* 15:814-817 (Jun. 1997).

Other Reference Publication (16):

Jones, D.H. et al., "Oral delivery of Poly(lactide-co-glycolide) encapsulated vaccines," *Behring Inst. Mitt.* 98:220-228 (Feb. 1997).

Other Reference Publication (17):

Jones, D.H. et al., "Immune Responses Following Oral and Parental Administration of Plasmid DNA Encapsulated in Poly(lactide-co-glycolide) Microparticles," *Int. Meeting on Nucleic Acid Vaccines*, Bethesda, MD, Abstract and attached Figures 2 and 3 and Figure entitled Stool IgA Response to PLG-Encapsulated DNA (Feb. 5-7, 1996).

Other Reference Publication (18):

Jones, D.H. et al. "Oral Delivery of Micro-Encapsulated DNA Vaccines," *Dev. Biol. Stand.* 92:149-155 (1998).

Other Reference Publication (19):

Jones, D.H. et al., "Protection of mice from *Bordetella pertussis* respiratory infection using microencapsulated pertussis fimbriae," *Vaccine* 13:675-681 (May 1995).

Other Reference Publication (20):

Jones, D.H. et al., "Poly(lactide-co-glycolide) microencapsulation of vaccine antigens," *J. Biotech.* 44:29-36 (1994).

Other Reference Publication (21):

Kreuter, J. "Nanoparticles and microparticles for drug and vaccine delivery," *J. Anat.* 189:503-505 (1996).

Other Reference Publication (25):

Puyal, C. et al., "A new cationic liposome encapsulating genetic material. A potential delivery system for polynucleotides," *Eur. J. Biochem.* 228:697-703 (1995).

Other Reference Publication (39):

Etlinger, H. "Carrier sequence selection-one key to successful vaccines," Imm. Today 13:52-55 (1992).

Other Reference Publication (44):

Morris, W. et al., "Potential of polymer microencapsulation technology for vaccine innovation," Vaccine 12:5-11 (1994).

Other Reference Publication (48):

Whalen, "DNA vaccines for emerging infectious diseases," Emerging Infect. Dis. 2:168-175 (1996).

(FILE 'HOME' ENTERED AT 15:39:54 ON 17 MAR 2003)

FILE 'MEDLINE, EMBASE, CANCERLIT, BIOTECHDS, BIOSIS, CAPIUS' ENTERED AT
15:41:07 ON 17 MAR 2003

L1 87723 S ENCAPSULAT?
L2 3564 S CATIONIC LIPID OR CATIONIC AMPHIPHILE OR CATIONIC LIPOSOME
L3 141 S L1 AND L2
L4 3110460 S DNA OR NUCLEIC OR PLASMID OR POLYNUCLEOTIDE
L5 91 S L4 AND L3
L6 49 DUP REM L5 (42 DUPLICATES REMOVED)

=>

L6 ANSWER 47 OF 49 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
AN 1988-00661 BIOTECHDS
TI Lipofection: a highly efficient, lipid-mediated **DNA**
-transfection procedure;
animal cell transfection using liposome **encapsulated**
DNA
AU Felgner P L; Gadek T R; Holm M; Roman R; Chan H W; Wenz M
CS Syntex
LO Institute of Bio-organic Chemistry, Syntex Research, 3401 Hillview
Avenue, Palo Alto, CA 94303, USA.
SO Proc.Natl.Acad.Sci.U.S.A.; (1987) 84, 21, 7413-17
CODEN: PNASA6
DT Journal
LA English
AB The uptake of exogenous **DNA** by eukaryotic cells is a very
inefficient process. Existing transfection methods all suffer from 1 or
more problems related to either cellular toxicity, poor reproducibility,
inconvenience or inefficiency of **DNA** delivery. A **DNA**
transfection protocol was developed using a synthetic **cationic**
lipid, N-(1-(2,3-dioleyloxy)propyl)- N,N,N-trimethylammonium
chloride (DOTMA). Small unilamellar liposomes containing DOTMA interact
spontaneously with **DNA** to form **lipid-DNA** complexes
with 100% entrapment of the **DNA**. DOTMA facilitates fusion of
the complex with the plasma membrane of the animal cells in culture,
resulting in both uptake and expression of the **DNA**. The
practicality of the method was tested using pSV2cat transfection of COS-7
(ATCC CRL 1651) cells and CV1 cells. The optimal quantity of **DNA**
varied with cell types with a small inhibitory effect above the optimum
level. Such 'lipofection' was 5- to 100-fold more effective than either
the calcium phosphate or DEAE-dextran transfection techniques. (26 ref)

L6 ANSWER 33 OF 49 CAPLUS COPYRIGHT 2003 ACS
AN 1997:757071 CAPLUS
DN 128:39581
TI Cationic lipids for drug delivery
IN Kirpotin, Dmitri; Chan, Daniel C. F.; Bunn, Paul
PA Kirpotin, Dmitri, USA; Chan, Daniel C. F.; Bunn, Paul
SO PCT Int. Appl., 22 pp.

CODEN: PIXXD2

DT Patent

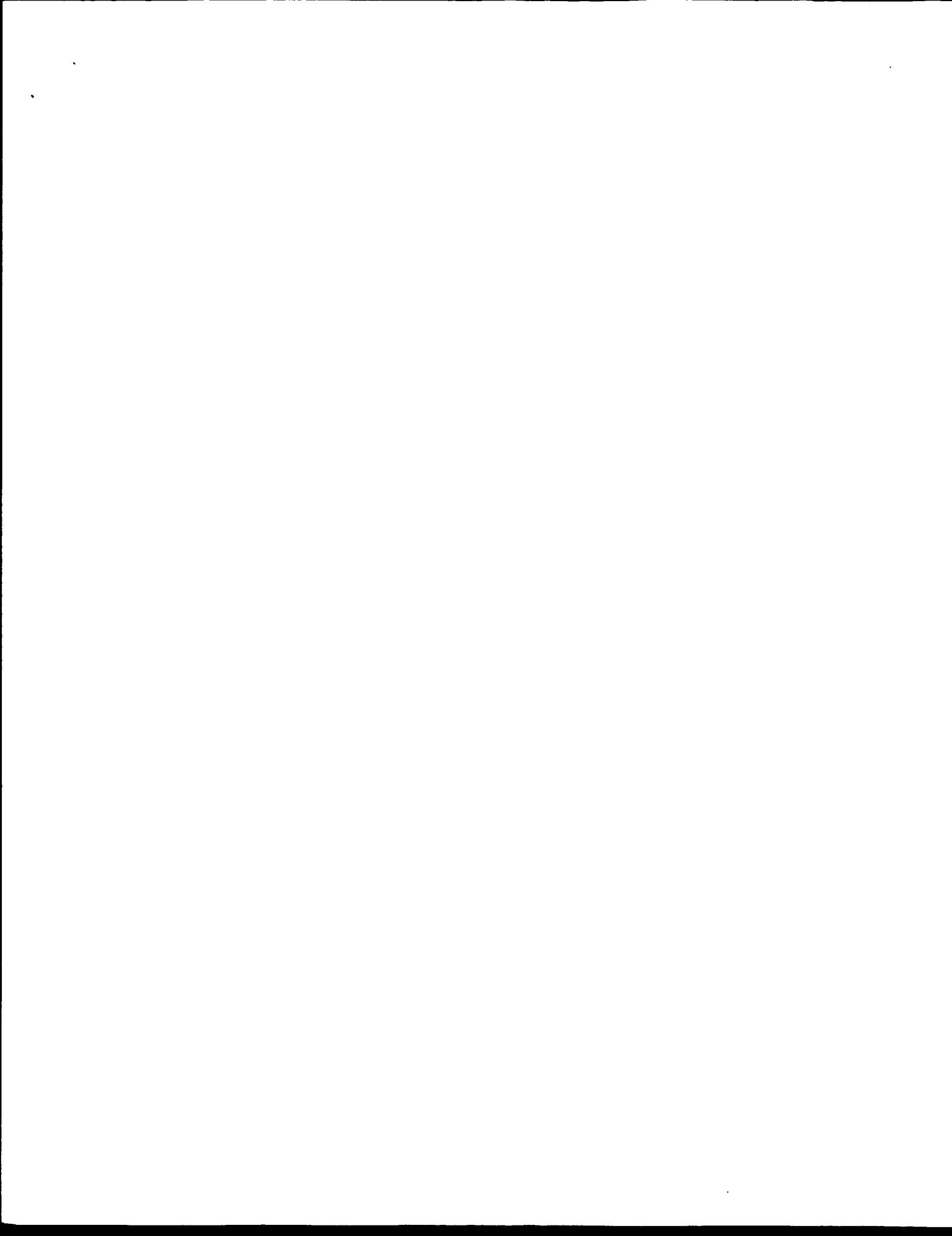
LA English

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9743363	A1	19971120	WO 1997-US8120	19970514
	W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
	US 5980935	A	19991109	US 1996-648558	19960515
	AU 9731248	A1	19971205	AU 1997-31248	19970514
	EP 923630	A1	19990623	EP 1997-926489	19970514
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
PRAI	US 1996-648558		19960515		
	WO 1997-US8120		19970514		
AB	The present invention relates generally to a non-toxic lipid conjugated with a cationic amino acid contg. a guanidino group. Specifically, the naturally-occurring lipid DOPE is combined with the naturally-occurring amino acid arginine. These compds. are useful for encapsulating and delivering pharmaceuticals and poly- and oligonucleotides. These compds. are composed of nontoxic and, in the case of Arg-DOPE, natural components, and therefore result in minimal undesirable effects. Methods for the use of cationic lipids are also claimed. N-L-arginyldioleoylphosphatidylethanolamine was prep'd. by the reaction of dioleoylphosphatidylethanolamine with N.alpha.-tert-butoxycarbonylarginine in the presence of N-ethyl-N-dimethylaminopropylcarbodiimide-HCl in CHCl ₃ . This compd. was formulated into aq. micellar solns.				

L6 ANSWER 31 OF 49 MEDLINE DUPLICATE 13
AN 1998115051 MEDLINE
DN 98115051 PubMed ID: 9452961
TI Cationic liposome--**plasmid DNA** complexes used for gene transfer retain a significant trapped volume.
AU Wasan E K; Fairchild A; Bally M B
CS Department of Advanced Therapeutics, B. C. Cancer Agency, Vancouver, Canada.. ewasan@bccancer.bc.ca
SO JOURNAL OF PHARMACEUTICAL SCIENCES, (1998 Jan) 87 (1) 9-14.
Journal code: 2985195R. ISSN: 0022-3549.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199802
ED Entered STN: 19980306
Last Updated on STN: 19980306.
Entered Medline: 19980226
AB The goal of this study is to determine whether cationic liposomes retain any trapped volume after their complexation to **plasmid DNA**. This serves two purposes: to further the understanding of the physical nature of liposome/**plasmid DNA** complexes used in gene therapy and to investigate the potential for codelivery of other **encapsulated** molecules with the liposome-**DNA** complexes. Cationic liposomes composed of N,N-dioleoyl-N,N-dimethylammonium chloride and dioleoylphosphatidylethanolamine (DODAC/DOPE, 50/50 mol %) **encapsulating** an aqueous trap marker were used to prepare liposome-**DNA** complexes at various charge ratios. The trapped volume before and after **DNA** binding was measured by two methods: dialysis and filtration. The effect of tissue culture medium on trapped volume was also investigated. A lipid-mixing assay was employed to further characterize the aggregation events that influence trap volume. The trapped volume (V_t) of neutral control liposomes was 1.1 +/- 0.04 microL/mumol, which was not affected by the addition of **DNA**. For cationic liposomes in the absence of **DNA** the V_t was 1.45 +/- 0.46 and 1.54 +/- 0.08 microL/mumol, as measured by the filtration and dialysis methods, respectively. After addition of **DNA**, the residual trapped volume (R_{Vt}) decreased to 0.43 +/- 0.1 microL/mumol and 0.47 +/- 0.05 microL/mumol, as determined by each method, respectively. R_{Vt} increased as the ratio of **cationic lipid** to **DNA** (nmol of lipid/mg of **DNA**) was increased above 10, a ratio that corresponds to a charge ratio (positively charged lipids to negatively charged phosphate groups) of 1.62. Aggregation and lipid-mixing were greatest at charge ratios coinciding with the lowest trapped volume. In the presence of tissue culture medium, the V_t of cationic liposomes but not neutral liposomes was reduced, suggesting that the salts have a direct effect on cationic liposomes in the absence of **DNA**. The R_{Vt} of both neutral and cationic liposomes in the presence of **DNA**, however, was not different from that of the liposomes in the absence of **DNA**. These results suggest that a significant trapped volume is retained by cationic liposomes after binding to **plasmid DNA**. This is an important finding with regard to the potential use of **DNA**/liposome complexes in the codelivery of other bioactive molecules at the time of cell transfection.

L6 ANSWER 20 OF 49 MEDLINE DUPLICATE 5
AN 2000220614 MEDLINE
DN 20220614 PubMed ID: 10758913
TI **Encapsulation of plasmid DNA in stabilized plasmid-lipid particles composed of different cationic lipid concentration for optimal transfection activity.**
AU Saravolac E G; Ludkovski O; Skirrow R; Ossanlou M; Zhang Y P; Giesbrecht C; Thompson J; Thomas S; Stark H; Cullis P R; Scherrer P
CS INEX Pharmaceuticals Corp., Burnaby BC, Canada.
SO JOURNAL OF DRUG TARGETING, (2000) 7 (6) 423-37.
Journal code: 9312476. ISSN: 1061-186X.
CY Switzerland
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200006
ED Entered STN: 20000613
Last Updated on STN: 20000613
Entered Medline: 20000601
AB In previous work (Wheeler et al. (1999) Gene Therapy 6, 271-281) we have shown that **plasmid DNA** can be entrapped in "stabilized **plasmid** lipid particles" (SPLP) using low levels (5-10 mol%) of **cationic lipid**, the fusogenic lipid dioleoylphosphatidylethanolamine (DOPE), and a polyethyleneglycol (PEG) coating for stabilization. The PEG moieties are attached to a ceramide anchor containing an arachidoyl acyl group (PEG-CerC20). However, these SPLP exhibit low transfection potencies *in vitro* as compared to **plasmid/cationic lipid** complexes formed with liposomes composed of cationic and neutral lipid at a 1:1 lipid ratio. The objective of this study was to construct SPLPs with increased **cationic lipid** contents that result in maximum transfection levels. A phosphate buffer detergent dialysis technique is described resulting in formation of SPLP containing 7-42.5 mol% DODAC with reproducible **encapsulation** efficiency of up to 80%. An octanoyl acyl group was used as anchor for the PEG moiety (PEG-CerC8) permitting a quick exchange out of the SPLP to further optimize the *in vitro* and *in vivo* transfection. We have demonstrated that this technique can be used to **encapsulate** either linearized **DNA** or supercoiled plasmids ranging from 3-20 kb. The SPLP formed could be isolated from empty vesicles by sucrose density gradient centrifugation, and exhibited a narrow size distribution of approximately 75 +/- 6 nm as determined by cryo-electron microscopy. The high **plasmid**-to-lipid ratio observed corresponded to one **plasmid** per particle. The SPLP consist of a lipid bilayer surrounding the **plasmid DNA** as visualized by cryo-electron microscopy. SPLP containing a range of DODAC concentrations were tested for *in vitro* and *in vivo* transfection. *In vitro*, in COS-7 cells transfection reached a maximum after 48 h. The transfection efficiency increased when the DODAC concentration in the SPLP was decreased from 42.5 to 24 mol% DODAC. Decreasing the **cationic lipid** concentration improved transfection in part due to decreased toxicity. *In vivo* studies using an intraperitoneal B16 tumor model and intraperitoneal administration of SPLP showed maximum transfection activity for SPLP containing 24 mol% DODAC. Gene expression observed in tumor cells was increased by approximately one magnitude as compared to **cationic lipid/DNA** complexes. The SPLP were stable and upon storage at 4 degrees C no significant change in the transfection activity was observed over a one-year period. Thus this phosphate buffer detergent dialysis technique can be used to generate SPLP formulations containing a wide range of **cationic lipid** concentrations to determine optimal SPLP composition for high transfection activity and low toxicity.



L6 ANSWER 12 OF 49 CAPLUS COPYRIGHT 2003 ACS
AN 2001:491059 CAPLUS
DN 136:58674
TI Spontaneous entrapment of polynucleotides upon electrostatic interaction with ethanol-destabilized cationic liposomes
AU Maurer, Norbert; Wong, Kim F.; Stark, Holger; Louie, Lenore; McIntosh, Deirdre; Wong, Tabitha; Scherrer, Peter; Semple, Sean C.; Cullis, Pieter R.
CS Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, BC, V6T 1Z3, Can.
SO Biophysical Journal (2001), 80(5), 2310-2326
CODEN: BIOJAU; ISSN: 0006-3495
PB Biophysical Society
DT Journal
LA English
AB This study describes the effect of ethanol and the presence of PEG lipids on the interaction of nucleotide-based polyelectrolytes with cationic liposomes. It is shown that preformed large unilamellar vesicles (LUVs) contg. a **cationic lipid** and a PEG coating can be induced to entrap polynucleotides such as antisense oligonucleotides and **plasmid DNA** in the presence of ethanol. The interaction of the cationic liposomes with the polynucleotides leads to the formation of multilamellar liposomes ranging in size from 70 to 120 nm, only slightly bigger than the parent LUVs from which they originated. The degree of lamellarity as well as the size and polydispersity of the liposomes formed increases with increasing **polynucleotide**-to-lipid ratio. A direct correlation between the entrapment efficiency and the membrane-destabilizing effect of ethanol was obsd. Although the morphol. of the liposomes is still preserved at the ethanol concns. used for entrapment (25-40%, vol./vol.), entrapped low-mol.-wt. solutes leak rapidly. In addn., lipids can flip-flop across the membrane and exchange rapidly between liposomes. Furthermore, there are indications that the interaction of the polynucleotides with the cationic liposomes in ethanol leads to formation of **polynucleotide-cationic lipid** domains, which act as adhesion points between liposomes. It is suggested that the spreading of this contact area leads to expulsion of PEG-ceramide and triggers processes that result in the formation of multilamellar systems with internalized polynucleotides. The high entrapment efficiencies achieved at high polyelectrolyte-to-lipid ratios and the small size and neutral character of these novel liposomal systems are of utility for liposomal delivery of macromol. drugs.

RE.CNT 59 THERE ARE 59 CITED REFERENCES AVAILABLE FOR THIS RECORD